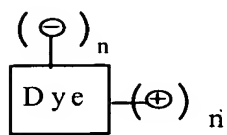
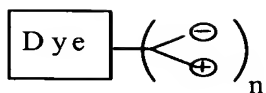




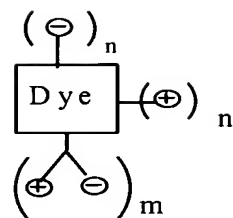
1/19



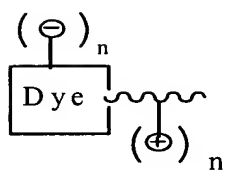
A



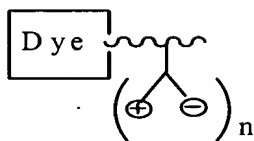
B



C

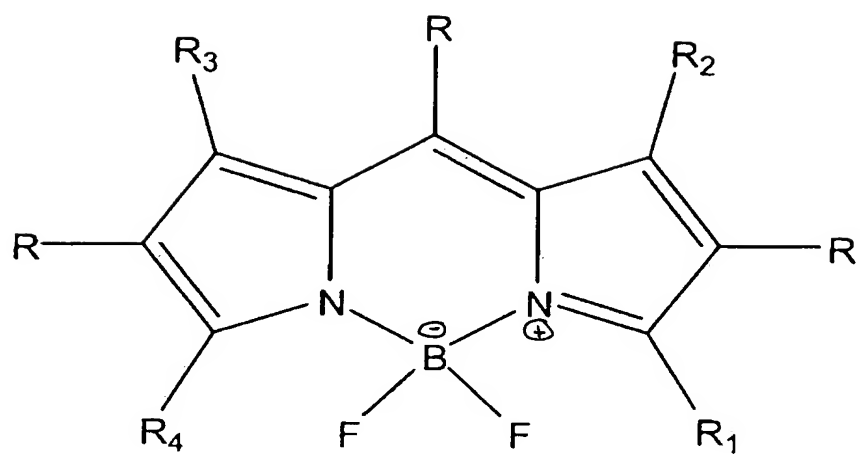


D



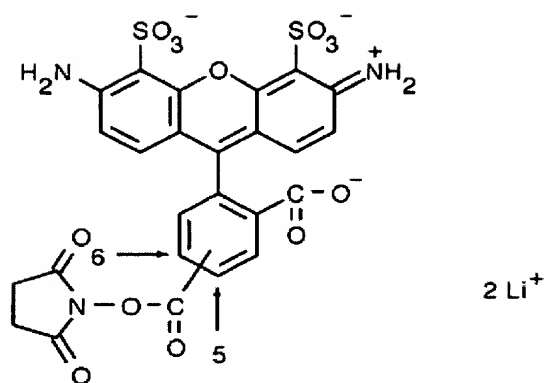
E

FIGURE 1



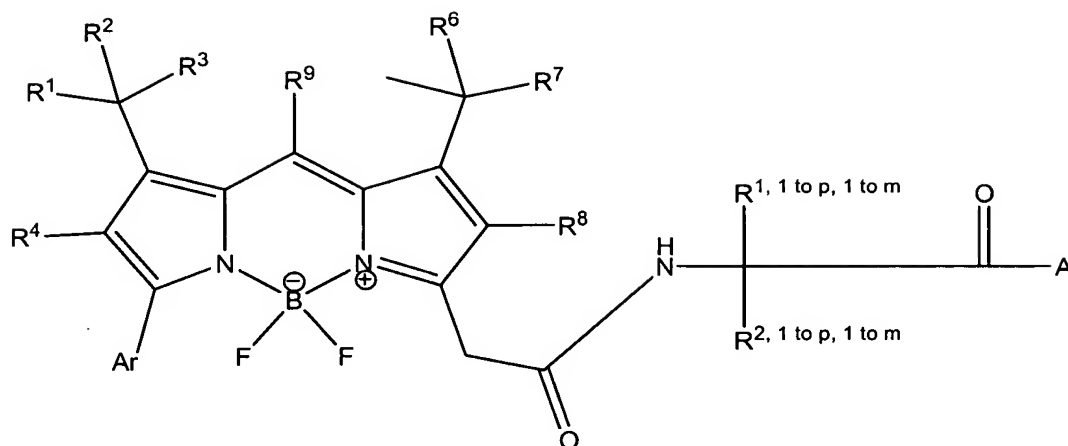
**BODIPY fluorophore, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene**

FIGURE 2



Alexa Fluor ® 488 carboxylic acid, succinimidyl ester dye structure

FIGURE 3



### General structure of an optical labeling molecule comprising a BODIPY dye moiety

A = Ester activator, NHCH<sub>2</sub>CH<sub>2</sub>SH, or other linker

R<sub>1</sub> to R<sub>9</sub> = to be defined

R<sub>1</sub>, 1 to p, 1 to m and R<sub>2</sub>, 1 to p, 1 to m = to be defined

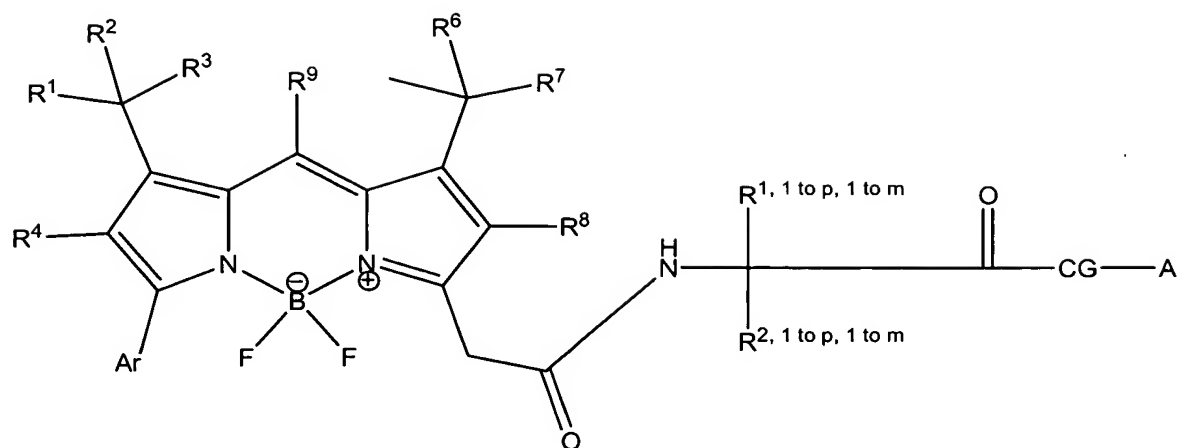
The R groups must be combined to have an equal number of non-titratable positive and negative groups to produce zwitterionic pairs

Ar = Aryl

r, n, m, p, q = 0, 1, 2, 3...

For each value of p, there are p values of m. These p values can be equal or different

FIGURE 4A



**General structure of an optical labeling molecule comprising a BODIPY dye moiety**

A = Ester activator, NHCH<sub>2</sub>CH<sub>2</sub>SH, or other linker

CG = Cleavable group

R<sup>1</sup> to R<sup>9</sup> = to be defined

R<sup>1</sup>, 1 to p, 1 to m and R<sup>2</sup>, 1 to p, 1 to m = to be defined

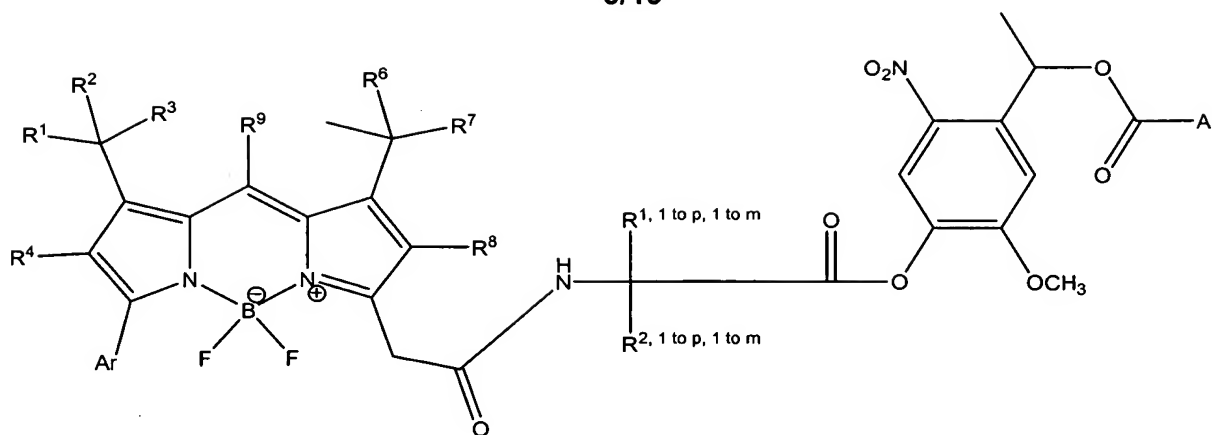
The R groups must be combined to have an equal number of non-titratable positive and negative groups to produce zwitterionic pairs

Ar = Aryl

r, n, m, p, q = 0, 1, 2, 3...

For each value of p, there are p values of m. These p values can be equal or different

**FIGURE 4B**



**General structure of an optical labeling molecule comprising a BODIPY dye moiety with a p-nitro anisole group**

A = Ester activator, NHCH<sub>2</sub>CH<sub>2</sub>SH, or other linker

R1 to R9 = to be defined

R1, 1 to p, 1 to m and R2, 1 to p, 1 to m = to be defined

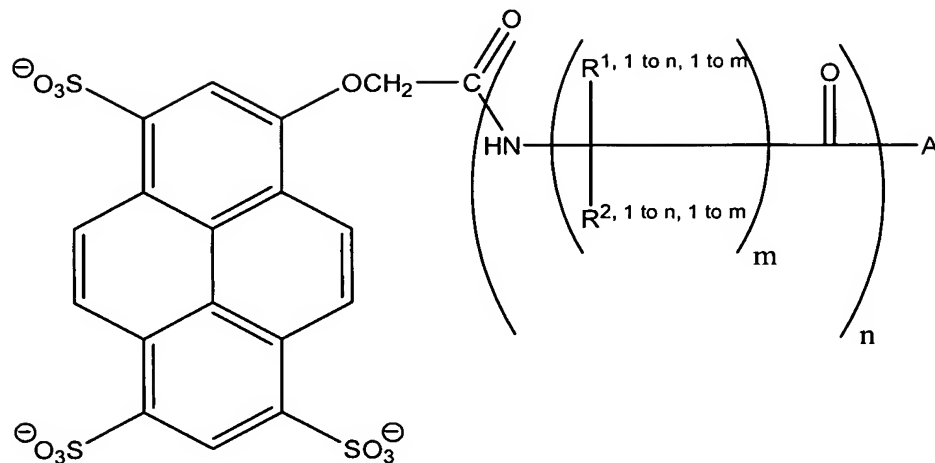
The R groups must be combined to have an equal number of non-titratable positive and negative groups to produce zwitterionic pairs

Ar = Aryl

r, n, m, p, q = 0, 1, 2, 3...

For each value of p, there are p values of m. These p values can be equal or different

FIGURE 5



**General structure of an optical labeling molecule comprising a Cascade Blue dye moiety**

$n, m = 1, 2, 3, \dots$

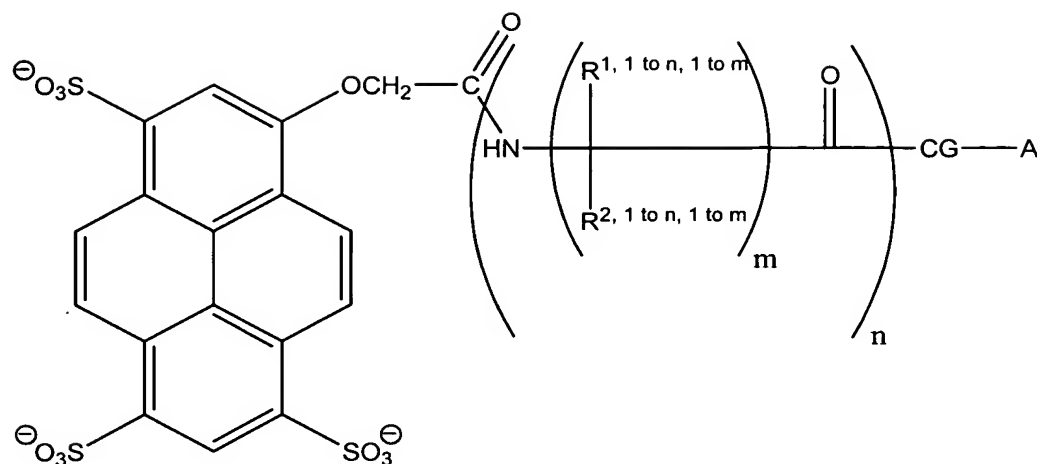
$\text{R}^1, 1 \text{ to } n, 1 \text{ to } m$  and  $\text{R}^2, 1 \text{ to } n, 1 \text{ to } m =$  to be defined

Three non-titratable cationic groups must be included in the R groups

A = nucleophilic attack activator

For each value of  $n$ , there are  $n$  values of  $m$ . These  $n$  values can be equal or different

**FIGURE 6A**



**General structure of an optical labeling molecule comprising a Cascade Blue dye moiety**

$n, m = 1, 2, 3, \dots$

$\text{R}^1, 1 \text{ to } n, 1 \text{ to } m$  and  $\text{R}^2, 1 \text{ to } n, 1 \text{ to } m =$  to be defined

Three non-titratable cationic groups must be included in the R groups

CG = cleavable group

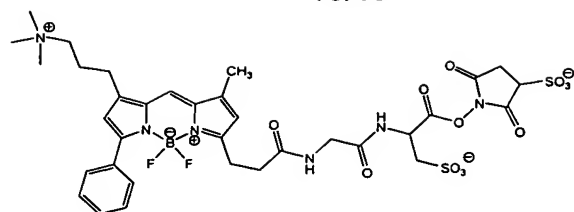
A = nucleophilic attack activator

For each value of  $p$ , there are  $p$  values of  $m$ . These  $p$  values can be equal or different

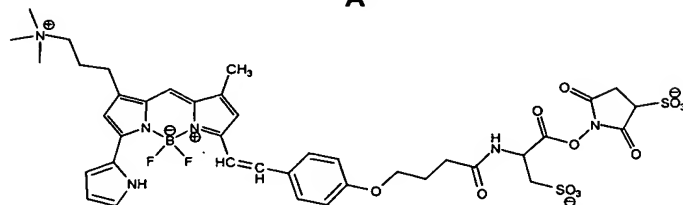
**FIGURE 6B**

FIGURE 7

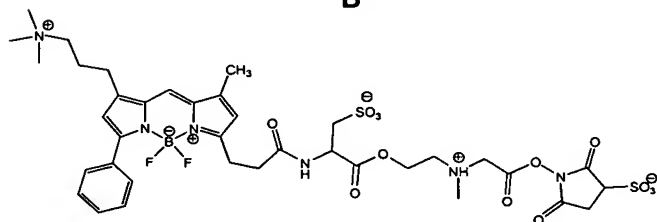
10/19



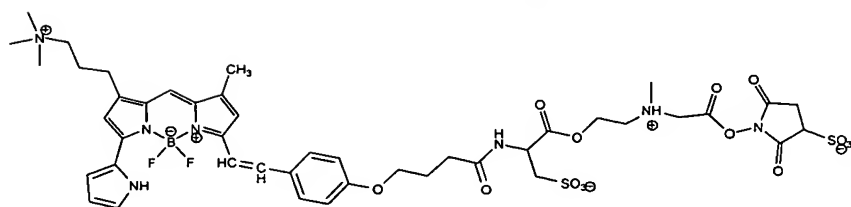
**A**



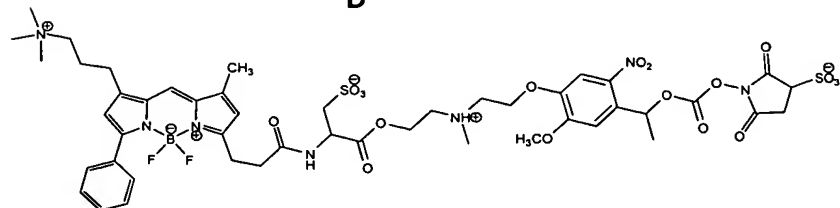
**B**



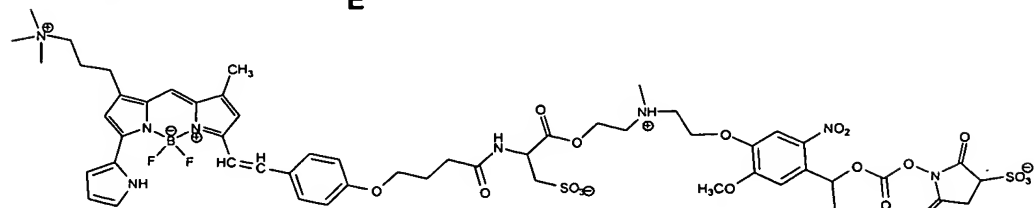
**C**



**D**



**E**



**F**

FIGURE 8A

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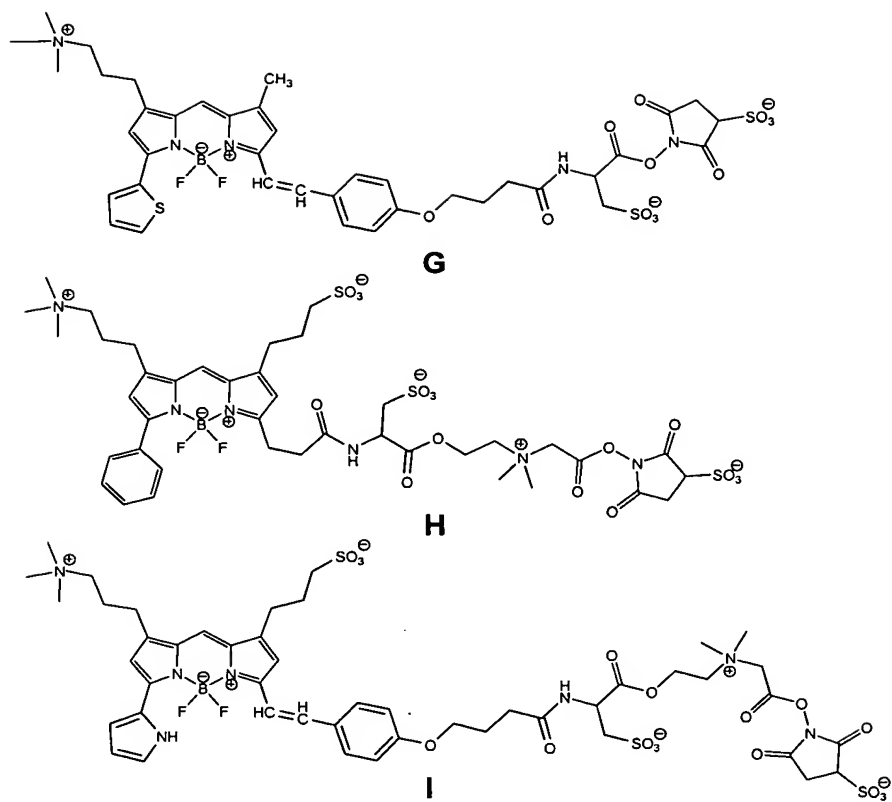
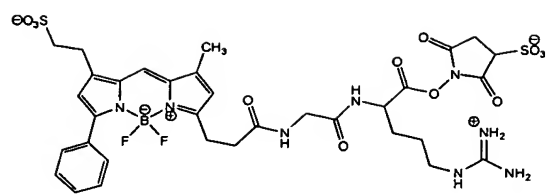
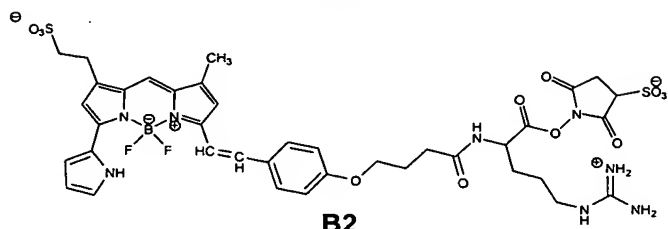


FIGURE 8B

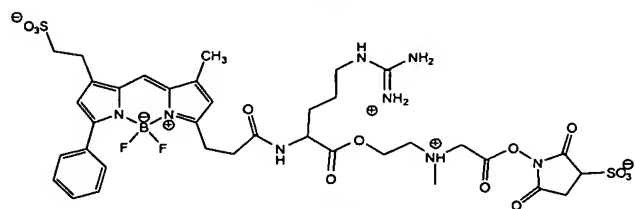
12/19



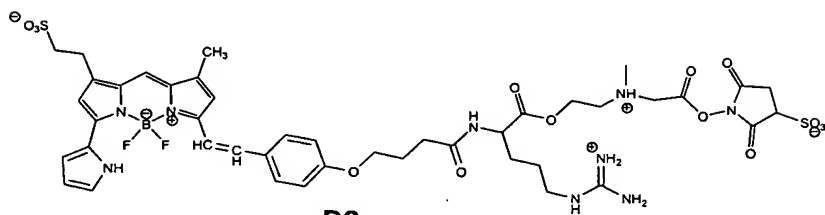
**A2**



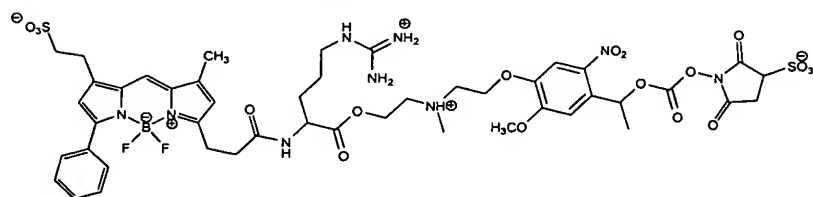
**B2**



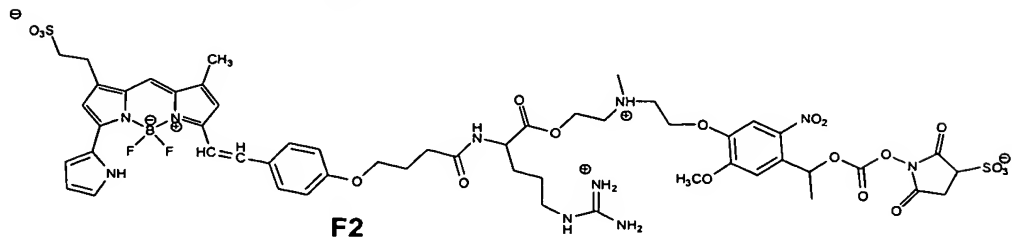
**C2**



**D2**



**E2**



**F2**

**FIGURE 9A**

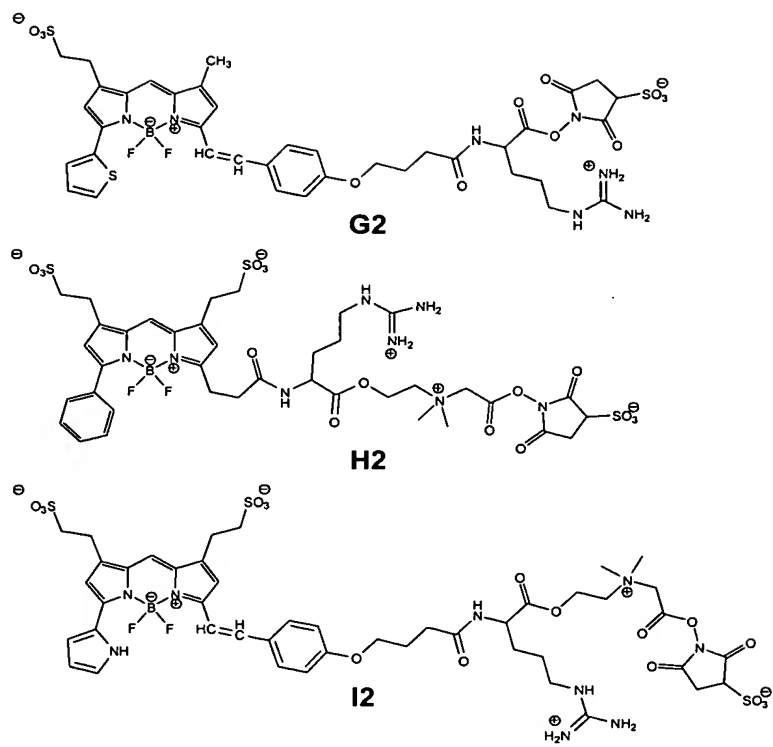


FIGURE 9B

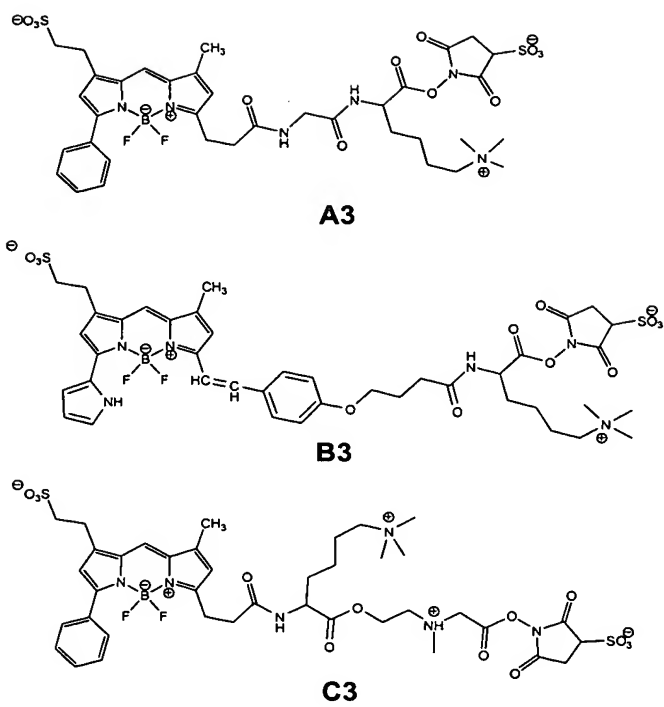
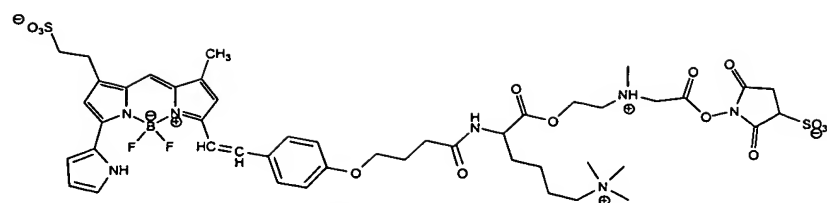
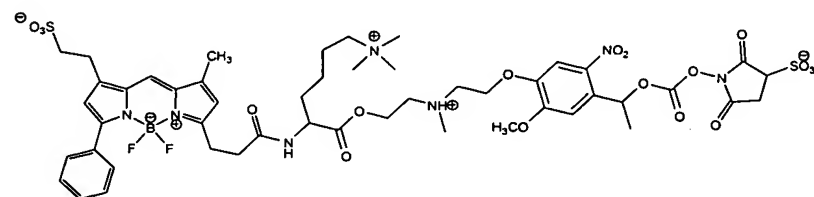
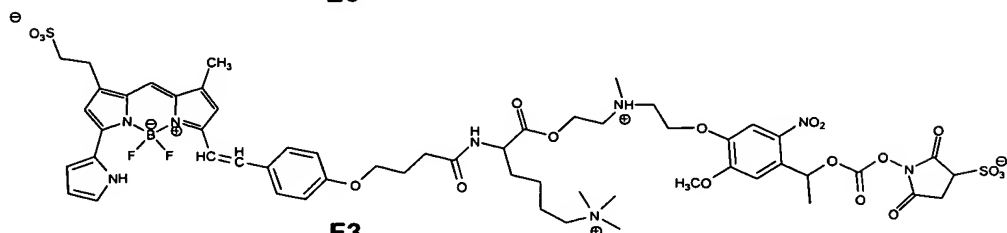
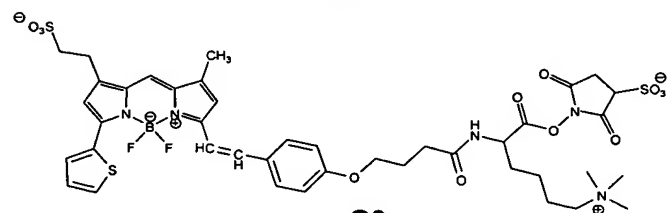
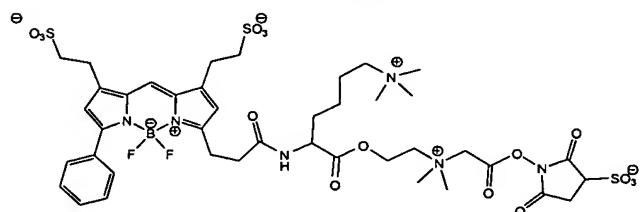
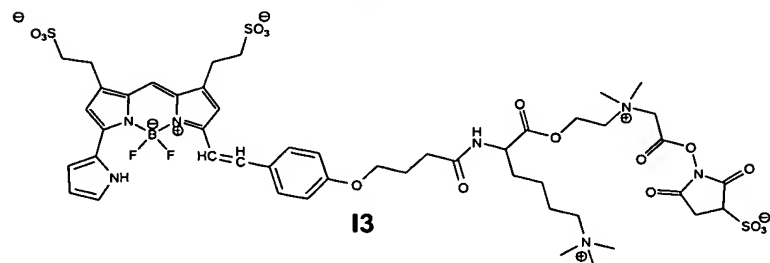
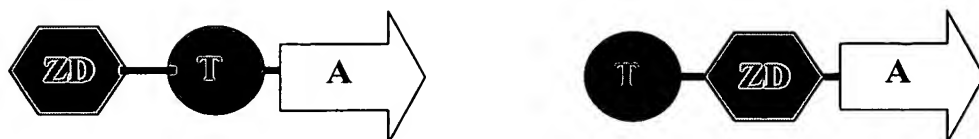


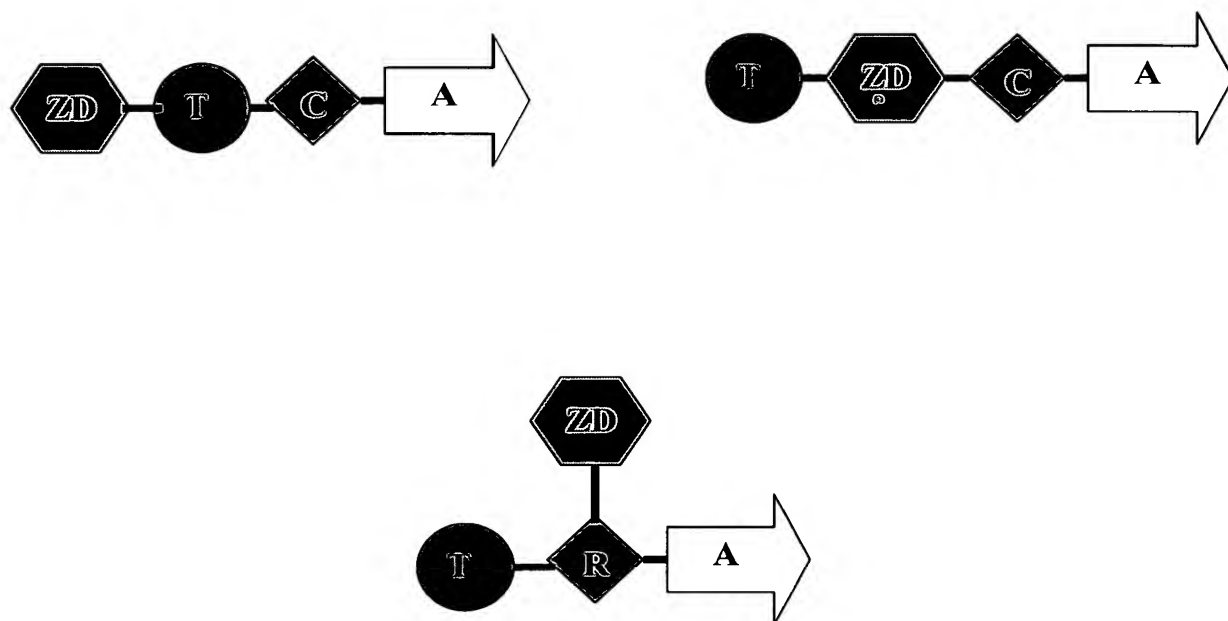
FIGURE 10A

**D3****E3****F3****G3****H3****I3****FIGURE 10B**



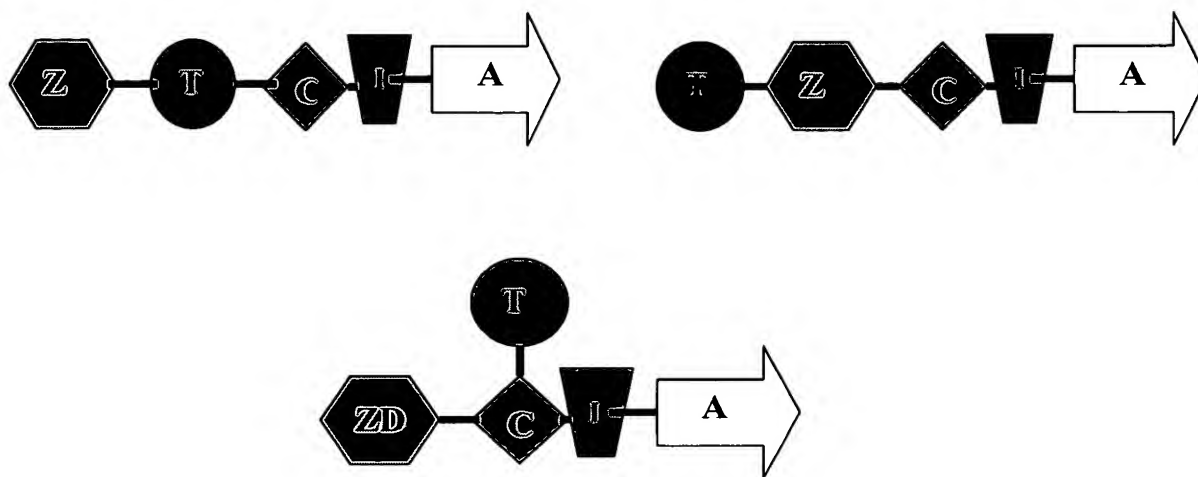
General structure of an optical labeling molecule wherein ZD is the zwitterionic dye moiety, T is the titratable group moiety, and A is the functional linker.

FIGURE 11



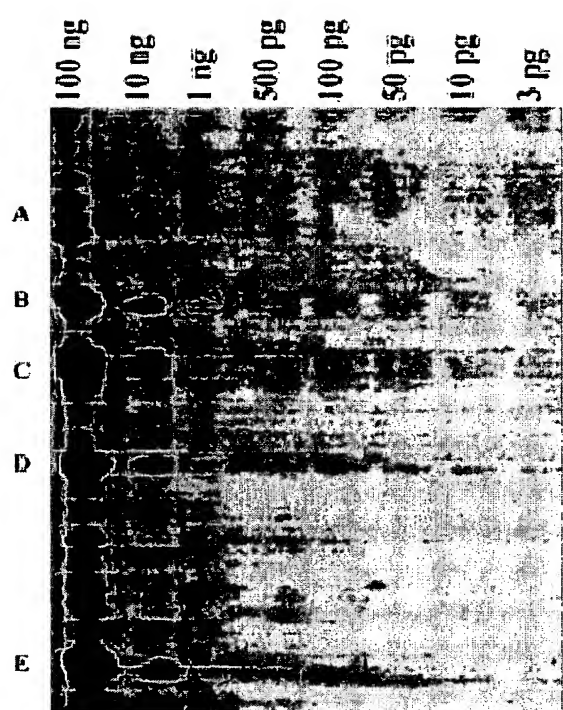
General structures of an optical labeling molecule wherein ZD is the zwitterionic dye moiety, T is the titratable group moiety, C is the cleavable moiety and A is the functional linker.

FIGURE 12



General structures of an optical labeling molecule wherein ZD is the zwitterionic dye moiety, T is the titratable group moiety, C is the cleavable moiety, I is the stable isotope moiety and A is the functional linker.

FIGURE 13



Gel showing the detection sensitivity obtained by prelabeling a set of standard proteins in SDS using a BODIPY dye from Molecular Probes

FIGURE 14